New Insights Into the Role of Tissue Eosinophils in the Progression of Colorectal Cancer: A Literature Review

Novas Perspetivas Sobre o Papel dos Eosinófilos Tecidulares na Progressão do Cancro Colorretal: Uma Revisão da Literatura

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ABSTRACT

Introduction: Amongst the inflammatory cells implicated in the immune surveillance of colorectal cancer, a growing body of evidence suggests a role for eosinophils in carcinogenesis. We aimed to review the value of tumour-associated tissue eosinophilia (TATE) in the prognosis of colorectal cancer emphasizing the identification and measurement of tissue-infiltrating eosinophils and their association with the clinicopathological features of the disease.

Material and Methods: We used PubMed and Web of Science search engines to retrieve studies that looked at the association between tissue eosinophils and colorectal cancer prognosis.

Results: We selected 15 studies for our review. In the majority of the studies, eosinophils were identified in hematoxylin-eosin stained sections and scores were generated for analysis. Most of the studies pointed to tumour-associated tissue eosinophilia as a favourable prognostic marker in colorectal cancer and found an inverse association between eosinophil count and the metastatic potential of these neoplasms. The association between tumour-associated tissue eosinophilia and established prognostic markers of colorectal cancer was assessed in some studies, with inconsistent results. Additionally, tumour-associated tissue eosinophilia decreased with the adenoma-carcinoma progression of colorectal lesions.

Discussion: Several mechanisms have been proposed regarding eosinophil chemoatraction to tumour tissues and eosinophil-cancer cell cross-talk, suggesting that eosinophils are actively involved in colorectal cancer progression. Although a scoring system is still lacking, tumour-associated tissue eosinophilia meets the criteria of a convenient histopathological prognosticator in colorectal cancer.

Conclusion: Collectively, current evidence associates the presence of eosinophils in the colorectal cancer microenvironment with the modulation of tumour progression. The clinical impact of this finding deserves future research.

Keywords: Colorectal Neoplasms; Eosinophils; Precancerous Conditions; Prognosis

RESUMO

Introdução: O papel dos eosinófilos na carcinogénese colorretal tem sido discutido em várias publicações científicas. O objetivo deste estudo foi o de rever o valor dos eosinófilos tecidulares no prognóstico do cancro colorretal, enfatizando a sua identificação, mensuração e associação com as características clinicopatológicas da doença.

Material e Métodos: Utilizámos os motores de busca PubMed e Web of Science para pesquisar estudos que associassem os eosinófilos tecidulares com o prognóstico do cancro colorretal.

Resultados: Selecionámos 15 estudos para a nossa revisão. Maioritariamente, a análise do infiltrado foi realizada através da coloração de hematoxilina-eosina, com criação de scores. A maioria dos trabalhos descreveu a eosinofilia tecidular como um fator de prognóstico favorável no cancro colorretal e estabeleceu uma associação inversa entre ela e o comportamento metastático dos tumores. A associação com outros fatores de prognóstico foi por vezes abordada, com resultados inconsistentes. A eosinofilia tecidular diminuiu na progressão adenoma-carcinoma.

Discussão: Vários mecanismos têm sido propostos para explicar a quimiotaxia de eosinófilos para os tecidos tumorais e a sua interação com as células neoplásicas, sugerindo o envolvimento dos eosinófilos na progressão do cancro colorretal. Apesar de não existir um sistema de avaliação validado, a eosinofilia tecidular associada a tumores pode constituir um parâmetro histopatológico de prognóstico no cancro colorretal.

Conclusão: As evidências disponíveis associam a presença de eosinófilos no microambiente do cancro colorretal com a modulação da sua progressão. O impacto clínico deste achado deve ser estudado no futuro.

Palavras-chave: Eosinófilos; Lesões Pré-Cancerosas; Neoplasias Colorretais; Prognóstico

INTRODUCTION

Colorectal cancer (CRC) is a leading neoplasia and a major cause of cancer mortality worldwide,[1] whose incidence has increased in recent years. Thus, great efforts have been developed to deepen our understanding of the disease. The identification of new prognostic factors and predictors of disease progression is of particular interest, since therapeutic options offered to CRC patients may depend on them.

The tumour, node, metastasis (TNM) stage system of the American Joint Committee on Cancer (AJCC) is the strongest prognosticator and the major determinant for management of patients diagnosed with CRC.[2] However, for

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intermediate stages of the disease, the TNM classification loses strength since for some patients with stage II CRC, complete surgical excision may be curative, but for other patients with the same stage, adjuvant therapies may be necessary.[3] Therefore, additional risk assessment is of utmost importance in these patients.[3]

Different histopathological prognostic markers have been assigned to CRC patients, namely histomorphological variants of CRC, tumour differentiation, lymphatic and venous invasion, perineural invasion, tumour budding, tumour necrosis and inflammatory response.[4] Importantly, novel prognostic markers must not only be accurate, but also easily accessible on hematoxylin-eosin stained specimens and broadly applicable.[4] In this context, correlates of inflammatory responses may prove of interest.

Several authors have reviewed the value of local inflammatory reaction in CRC prognosis. Cells of both the innate and the adaptive immune systems are found in infiltrating CRCs, with a dual effect on the stimulation and inhibition of CRC growth.[5] Overall tissue inflammatory infiltration has been considered as a favourable prognostic marker.[4,6] Amongst the specific types of inflammatory cells implicated in CRC prognosis, T lymphocytes have been related both to positive and negative outcomes, depending on the subset considered.[5,6] Importantly, it is well established that CD3+ and CD8+ T cells infiltrates are related to good prognosis.[5-7] Concerning macrophages, the results are also contradictory since M1 and M2 macrophages are associated with tumour growth inhibition and stimulation, respectively.[5,8] However, CD68+ macrophages have been linked to a favourable outcome.[4,6] Neutrophils, mast cells and dendritic cells have been also implicated in CRC prognosis to a lesser extent.[5,6]

Eosinophilic infiltration of tumour tissues, also called tumour-associated tissue eosinophilia (TATE), has been referred as an easily recognizable histological parameter, related with survival and recurrence of CRC.[4] Several studies reported TATE in CRC as an independent prognostic factor. However, the role of eosinophils in colorectal carcinogenesis is still not fully understood. Therefore, a review of the literature in this area of study may have a great impact on the understanding of previously published data. Furthermore, it will certainly raise awareness as to the importance of these immune cells in the context of future research studies. In the present work, we aimed to review the value of TATE in the prognosis of CRC, highlighting the identification and measurement of tissue-infiltrating eosinophils and the association of TATE with clinicopathological features and prognostic outcomes in patients diagnosed with CRC.

MATERIAL AND METHODS

We performed a literature search using PubMed and Web of Science, for articles published until June 27, 2017. Regarding the search strategy and inclusion criteria for the main purposes of our review, the search terms were 'colorectal cancer AND eosinophils'. Only articles published in English were considered. No limits were applied concerning the year of publication. Review articles and unavailable abstracts were excluded. After scanning the titles and abstracts of the retrieved studies, we selected the full text of 15 original research articles in accordance with the objectives established and all articles were included in our review.[9-23] Data from the included studies were independently extracted from each study and comprised: author; year of publication; sample size; participants' characteristics (mean age and gender proportion); follow-up assessment; tumour classification, stage and clinicopathological features; TATE characterization (i.e. staining, count and location); and major findings. In order to enable a better understanding of our work, we performed a broad search of the literature on the biology of eosinophils and their role in cancer, as well as on CRC up-to-date information, prognosis, microenvironment and inflammation. We included the most significant studies in the final reference list.

RESULTS

Baseline characteristics of the selected studies

We incorporated 15 original research articles in our review, published between 1983 and 2016.[9-23] Twelve studies associated TATE with survival and/or metastatic behaviour of CRC[9,11,12,14-16,18-23] and three studies associated TATE with malignant progression of colorectal lesions.[10,13,17] The first set of studies evaluated 2523 CRCs.[9,11,12,14-16,18-23] The mean age of the participants was recorded in seven studies and ranged between 62.1 and 73 years.[9,11,14,16,21-23] Nine studies detailed gender proportion, totalling 835 males and 1136 females.[9,11,12,14,16,18,21-23] Nine studies specified the CRC staging approach: TNM was used in four studies[9,11,12,15] and Dukes staging was performed in five studies.[14,16,18,20,23] The median follow-up interval was recorded in five studies and ranged from 35.4 to 105 months.[9,11,12,15,18] In one study, the average time that patients were in the study was 73 months.[20] In other studies, the follow-up period was 60 months or longer.[14,16] Luebbers *et al* assessed survival data for periods between one and 47 months.[21] Pretlow *et al* recorded survival data for a period of 11 months for all patients, although for the majority of the patients included in their study, the follow-up period was18 months.[23] The second set of three studies comprised a total of 986 colorectal benign and malignant lesions.[10,13,17] In these studies, no information was recorded concerning the mean age of participants and gender proportion.[10,13,17] A summary of the selected studies is presented in Table 1.

TATE characterization

In seven of the studies herein included, eosinophils were identified in hematoxylin-eosin stained sections.[10-13,16-18] In one study, eosinophils were assessed by immunohistochemistry for eosinophil peroxidase (clone 144B, homebrew from Dr. James Lee, Mayo Clinic, Arizona).[9] In another study, the authors included both methods for eosinophil staining, applying the antibody EG-2 (Anti-Human ECP/EPX, Pharmacia Upjohn, Uppsala, Sweden) in immunohistochemistry technique.[15] In three

Table 1 – Summary of the selected studies

First author, year of publication	Sample size	Patients		Median follow-up (months)	Tissue samples		
		Mean age of participants (years)	Gender proportion (male/female)		Tumour classification	Tumour staging	Tumour stages
Prizment, 2016[9]	441	73	0/441	104.4	Colorectal cancer	TNM	NR
Cho, 2016[10]	50	NR	NR	NA	Tubular adenoma (low and high grade dysplasia) Adenocarcinoma	NR	NR
Harbaum, 2015[11]	381	68.5	166/215	45	Adenocarcinoma Others	TNM	I-IV
Richards, 2012[12]	130	68% of patients ≥ 65	68/62	105	Colorectal cancer	TNM	I-III
Kiziltaş, 2008[13]	448	NR	NR	NA	Hyperplastic polyp Serrated adenoma Tubular adenoma Tubulovillous adenoma Villous adenoma Adenocarcinoma	NR	NR
Nespoli, 2004[14]	192	65.6 and 62.1*	105/87	60†	Colorectal adenocarcinoma	Dukes	B and C
Nagtegaal, 2001[15]	160	NR	NR	35.4	Rectal adenocarcinoma	TNM	I-IV
Fernández-Aceñero, 2000[16]	126	67.4	70/56	60 (minimum)	Colorectal carcinoma, excluding signet ring and mucinous variants	Dukes	A-C
Moezzi, 2000[17]	488	NR	NR	NA	Hyperplastic polyp Tubular adenoma Tubulovillous adenoma Villous adenoma Intramucosal carcinoma Invasive adenocarcinoma	NR	NR
Nielsen, 1999[18]	584	70 (median)	344/240	61	Colorectal cancer	Dukes	A-D
McGinnis, 1989[19]	61	NR	NR	NA	Colonic carcinoma	NR	NR
Fisher, 1989[20]	331	NR	NR	73‡	Rectal carcinoma	Dukes	A-D
Luebbers, 1985[21]	24	62.7	19/5	1 to 47§	Colorectal carcinoma	NR	NR
Pretlow, 1984[22]	26	63.9	21/5	NA	Colorectal carcinoma	NR	NR
Pretlow, 1983[23]	67	65.3	42/25	11 and 18§	Colorectal carcinoma	Dukes	B and C

NR: not reported; NA: not applicable
* with and without postoperative infection, respectively; † follow-up period after surgery; ‡ average time on study; § range of follow-up periods

articles, eosinophils were identified by hematoxylin, eosin Y and azure II staining procedure.[19,21,22] Also, in two articles Giemsa staining was used for eosinophil recognition.[20,23] In one study, the staining procedure was not clearly reported, but presumably hematoxylin-eosin staining was used.[14]

Regarding eosinophil counts, most of the studies established scores based on the number of eosinophils in a particular area or the percentage of eosinophils relative to total stromal inflammatory cells (seven and two studies, respectively).[9,11-13,15-18,20] In five articles, scores were not applied.[10,19,21-23] In one study the authors considered three categories for analysis.[14]

The location of eosinophil counts within the lesions was reported in 13 studies.[9-15,17-22] In the study performed by Prizment *et al*, eosinophils were counted in the epithelial and stromal compartments.[9] Cho *et al* performed cell counts in the mucosa and submucosa, near the neoplastic lesion.[10] In three studies, intratumoural and peritumoural eosinophils were assessed.[11,13,15] Richards *et al*, Nespoli *et al* and Nielsen *et al* evaluated only peritumoural eosinophils.[12,14,18] In other three studies, the authors counted eosinophils in sections remote from the margin and contiguous to the margin, defined as "one cm from the border of tumour with uninvolved mucosa" and "the border itself", respectively.[19,21,22] In one study, the eosinophil count was performed in the stroma of the lesions; in invasive carcinoma cases, the authors assessed the transitional zone in particular ("the area between normal tissue and tumour").[17] In another article, the authors presented eosinophil counts in the "interface of the tumour and underlying tissues at the site of deepest penetration".[20]

A summary of TATE characterization described in the selected studies is presented in Table 2.

TATE as a favourable prognostic marker in CRC

In nine studies included in our review, survival analyses were performed in relation to TATE.[9,11,12,14-16,18,20,23] Table 3 highlights the association between TATE and prognostic outcomes within each study.

Briefly, TATE was associated with improved overall and/or CRC-specific survival in seven studies.[9,11,15,16,18,20,23] Further, TATE was inversely associated with all-cause and CRC-specific death in the study of Prizment *et al*.[9] TATE could also predict progression-free survival in one study[11] and relapse-free survival in another study.[16] Importantly, five studies pointed TATE as an independent prognostic factor in

Table 2 – Summary of methods described in the selected studies to achieve TATE characterization

First author, year of publication	TATE characterization		
	Staining	Count	Location
Prizment, 2016[9]	IHC	3 to 4 scores based on the No. of EØ / 0.28 mm^2	Epithelium and stroma
Cho, 2016[10]	HE	No. of EØ in 3 hotspots evaluated at HPF	Mucosa and submucosa near neoplastic lesion
Harbaum, 2015[11]	HE	4 scores based on the No. of EØ / 0.24 mm^2	Intratumoural and peritumoural
Richards, 2012[12]	HE	2 groups based on the median EØ count / 0.018 mm^2	Peritumoural
Kiziltaş, 2008[13]	HE	3 scores based on the percentage of EØ relative to total stromal inflammatory cells	Intratumoural and peritumoural
Nespoli, 2004[14]	NR (presumably, HE)	3 categories: absent, moderate or conspicuous	Peritumoural
Nagtegaal, 2001[15]	HE IHC	3 scores based on the No. of EØ / 2.1 mm^2	Intratumoural and peritumoural
Fernández-Aceñero, 2000[16]	HE	2 or 4 groups based on the No. of EØ / HPF	NR
Moezzi, 2000[17]	HE	3 scores based on the percentage of EØ relative to total stromal inflammatory cells	Stroma
Nielsen, 1999[18]	HE	4 groups based on the No. of EØ / 0.17 mm^2	Peritumoural
McGinnis, 1989[19]	Hematoxylin, eosin Y and azure II	No. of EØ / mm^2	Sections remote from the margin Sections contiguous to the margin
Fisher, 1989[20]	Giemsa	3 scores based on the No. of EØ in 30 fields (x1000)	Interface of the tumour and underlying tissues at the site of deepest penetration
Luebbers, 1985[21]	Hematoxylin, eosin Y and azure II	No. of EØ / mm^2	Sections remote from the margin Sections contiguous to the margin
Pretlow, 1984[22]	Hematoxylin, eosin Y and azure II	No. of EØ / mm^2	Sections remote from the margin Sections contiguous to the margin
Pretlow, 1983[23]	Giemsa	No. of EØ / mm^2	NR

TATE: tumour associated tissue eosinophilia; IHC: immunohistochemistry; No(s).: number(s); EØ: eosinophil(s); HE: hematoxylin-eosin; HPF: high power field; NR: not reported

CRC.[9,11,15,16,18] Also significant, in four studies, the prognostic value of TATE was maintained in intermediate stages of the disease - TNM stages II and III or Dukes stages B and C.[9,11,18,23] Two studies stated a lack of association between eosinophil count and cancer-specific or overall survival in univariate analysis.[12,14]

Association between TATE and metastatic behaviour of CRC

Nagtegaal *et al* described a significant association between high scores of eosinophils and lower rates of distant metastases ($p = 0.03$).[15] Additionally, an inverse relationship was observed between peritumoural eosinophils and local recurrence and distant metastases ($p = 0.007$ and $p = 0.009$, respectively).[15] Four other studies described significantly higher concentrations of eosinophils in tumours without metastases as compared to metastatic ones ($p < 0.05$).[19,21-23] In one of those studies, the proportion of tumours with less than 30 eosinophils / mm^2 with metastases was significantly higher than the proportion of tumours with more than 30 eosinophils / mm^2 with metastases.[23] Later on, the same group suggested a threshold lower than 30 eosinophils / mm^2.[22] In another study of the same group, cut-offs predictive of absence of metastases were proposed, namely 20 eosinophils / mm^2 and 25 eosinophils / mm^2, respectively depending on whether sections remote or contiguous to the margin of the tumour were considered.[19]

Association between TATE and established prognostic factors of CRC

Several items related to patients and tumour characteristics were analysed in association with TATE in the herein included studies. In this section, we summarize the association between TATE and the established prognostic factors of CRC, namely: stage, grade (differentiation), lymphatic, venous and perineural invasion, tumour budding

and inflammation.[4]

Higher eosinophil counts were significantly associated with lower CRC stage in four studies ($p \leq 0.04$).[9,11,15,20] In one study, the aforementioned association was not observed.[16]

In two studies, TATE was associated with better tumour differentiation ($p \leq 0.05$).[11,20] However, these data were not corroborated in four other studies.[9,15,16,22]

Increasing inflammatory reaction was positively associated with TATE in two studies ($p \leq 0.001$).[11,12] TATE was significantly correlated with several inflammatory cell types in different studies, namely CD8+, CD3+, CD4+, neutrophils, macrophages and mast cells.[9,15] However, in three studies, no association was found between TATE and mast cell counts.[18,20,23] The concentration of plasma cells was significantly related to the concentration of eosinophils in one study.[19]

In one study, a higher eosinophil count was significantly associated with the absence of lymphatic and venous invasion and tumour budding ($p \leq 0.02$).[11] However, Fernández-Aceñero *et al* did not find an association between TATE and vascular or neural invasion.[16]

TATE in adenoma-carcinoma progression of colorectal lesions

As stated before, three studies included in our review addressed the intensity of TATE in different colonic lesions, including hyperplastic polyps, adenomas and adenocarcinomas (Table 1).[10,13,17] Table 4 summarizes the results of these studies.

It was observed that the number of eosinophils significantly decreased throughout adenoma-carcinoma progression.[10,13,17] The intensity of TATE in hyperplastic polyps was significantly lower than in adenomatous lesions[13,17] and adenocarcinomas.[13] In the study of Kiziltaş *et al*, there was no significant difference in TATE intensity between low- and high grade dysplasia,[13] in line with the results of Cho *et al*.[10] Still, in both studies, the intensity of TATE was higher in high grade dysplasia compared to adenocarcinomas.[10,13] Additionally, Moezzi *et al* described a higher TATE in intramucosal carcinoma compared to invasive carcinoma.[17] Kiziltaş *et al* further included serrated adenomas in their series and found that TATE was lower in the hyperplastic polyps and higher in adenomatous polyps compared to those lesions ($p < 0.001$).[13]

DISCUSSION

Tumour microenvironment refers to the malignant and tumour-associated stromal and immune cells, as well as the cross-talk between them.[24] The immune system plays a dual action both in cancer promotion and prevention.[25] Considering the critical role of the immune system in the recognition and elimination of transformed cells - cancer immunosurveillance - new insights have emerged regarding the prognostic role of immune infiltrates in tumour tissues.[25] In this context, TATE has been widely studied, as previously revised.[4,6,26-31] In this study, we aimed at reviewing the prognostic value of TATE in CRC. In contrast to previous reviews, which summarized this topic in the context of wider subjects,[4,6,26,28,29,31] our review included a larger number of studies and provided more detailed information on TATE characterization and on the association between TATE and other clinicopathological features in CRC. Moreover, we reviewed the association of TATE and adenoma-carcinoma progression. Therefore, we accomplished a more exhaustive understanding of the published data.

TATE has been associated with a favourable prognosis in several types of solid tumours, including CRC.[4,6,26-29,31] Our analysis adds to this concept. Importantly, the prognostic value of TATE was maintained when TNM stages II and III of the disease were considered.[9,11] These findings support that the evaluation of TATE in CRC specimens may be useful for individual prognosis and might be used in the selection of patients who benefit from aggressive therapies as previously suggested.[4,16,18,29]

Despite the association found in different studies, the mechanisms by which eosinophils are recruited to the cancer niche and influence cancer prognosis remain unclear. However, current knowledge suggests that TATE plays an active and protective role in CRC progression rather than being an occasional finding. Understanding the molecular mechanisms involved in the cross-talk between eosinophils and CRC cells would certainly contribute to improve the existing knowledge of the immunogenic characteristics of the disease and may have a major impact in the development of novel therapeutic targets.[6,9,11,26,28,29,31-35]

In the case of CRC, eosinophil recruitment, activation and survival was suggested to be dependent on local production of IL-5 by tissue resident eosinophils[36] and active damage associated molecular pattern (DAMP) molecules, namely high mobility group box 1 (HMGB1), in the context of tumour cell necrosis.[32] Surprising findings have been achieved on the role of eotaxins in eosinophil chemoatraction to CRCs, since low numbers of tissue eosinophils are found both in primary CRCs[10,37] and liver metastases,[37] albeit not always associated with low levels of tissue eotaxins.[37,38] The contribution of neoplastic epithelial cells for the secretion of eotaxins appears to be residual.[10,39] Briefly, three mechanisms may explain the lower number of eosinophils in CRC tissues despite the often high levels of tissue eotaxins: the eosinophil chemoattractant role of eotaxins may be blocked in CRC[37]; lower levels of eotaxins in plasma may have impact in eosinophil traffic to the tissues[38,39]; or the differential eotaxins compartmentalization in CRC tissues influences eosinophil chemoatraction.[10]

Following activation, several mechanisms for CRC control by eosinophils have been proposed. After attachment to carcinoma cells, eosinophils lose their IL-5 transcripts and undergo apoptosis, releasing their cytotoxic granules.[36] Eosinophil degranulation results in the release of major basic protein (MBP) and eosinophil peroxidase (EPO), which promote oxidation of cancer cell lysates, both directly and indirectly through neutrophils.[32] Additionally, the cytotoxic effect of human eosinophils, inducing both apoptosis and necrosis, against intestinal carcinoma cell line Colo-205 was

Table 3 – Association between TATE and prognostic outcomes

First author, year of publication	Outcome measured	Statistical methods
Prizment, 2016[9]	- 5-year all-cause and CRC survival - 5-year all-cause and CRC-specific death - Total follow-up all-cause and CRC-specific death	- Kaplan-Meier plots and log-rank tests - Cox proportional hazards regression
Harbaum, 2015[11]	- Progression-free survival - Cancer specific survival	- Kaplan-Meier and log-rank tests - Cox proportional hazards regression
Richards, 2012[12]	- Cancer-specific survival	- Kaplan-Meier - Cox proportional hazards regression
Nespoli, 2004[14]	- 5-year overall survival	- Kaplan-Meier and log-rank tests - Cox proportional hazards regression
Nagtegaal, 2001[15]	-Cumulative survival (2-year) - Local recurrence rate (2 year) - Distant metastases rate (2year)	- Kaplan-Meier and log-rank tests - Cox proportional hazards regression
Fernández-Aceñero, 2000[16]	- Overall survival - Relapse-free survival	- Kaplan-Meier and log-rank tests - Cox proportional hazards regression
Nielsen, 1999[18]	- Overall survival	- Kaplan-Meier and log-rank tests - Cox proportional hazards regression
Fisher, 1989[20]	- Overall survival	- Life table plots - Mantel-Cox statistic
Pretlow, 1983[23]	- Survival at 18 months	- Wilcoxon 2-sample test - Fisher's exact test

TATE: tumour associated tissue eosinophilia; CRC: colorectal cancer; EØ: eosinophil(s); HR: hazard ratio; CI: confidence interval; No(s).: number(s)

assigned to eosinophil cationic protein, eosinophil derived neurotoxin (EDN), TNF-α, and granzyme A, depending on CD11a/CD18 for effector-target cell adhesion, and γδTCR/CD3.[40,41] The same group addressed IL-18 as a key mediator of eosinophils-Colo-205 cells contact, through CD11a and ICAM-1 adhesion molecules, contributing to the pro-apoptotic action of eosinophils.[33] Conversely, Taylor *et al* previously stated that CD11a was not involved in eosinophil binding to MCA-38 colon adenocarcinoma cells, but the authors used mouse eosinophils.[42] Still, they attributed a direct cytotoxic effect of eosinophils against MCA-38 cells, demonstrating the role of protein tyrosine kinase and cyclic AMP in effector-target cell adhesion.[42] Earlier, the local anti-tumour effect of IL-4 (secreted by engineered colon 26 cells) was endorsed in part to eosinophils in an early stage, supporting an advantageous role of TATE in the defence against cancer.[34] Recently, a protective role was also attributed to IL-18 in a mouse model of inflammatory colon cancer and inflammatory bowel disease (AOM/DSS mice).[39] The levels of IL-18 were likely related with TATE, rather than with epithelial cells secretion.[39] IL-17E (IL-25) was found to increase TATE and inhibited tumour growth, through IL-5 induction, in a human colon tumour xenograft model.[35] Thus, eosinophils may be engaged in the anti-tumour effect of IL-17E, and IL-17E was endorsed as a putative effective therapy in colon adenocarcinoma.[35]

In addition to the aforementioned molecular mechanisms, the relation between eosinophils and other immune cells raises the hypotheses that eosinophils may also exert their action via other cells and vice-versa.[9,15,19] The interaction between eosinophils and other immune cells has been recognized.[26-28,31,43] Interestingly, Nagtegaal *et al* proposed a model for protective immune responses in rectal cancer, in which T cells interact with nonspecific immune response (eosinophils, neutrophils, mast cells, macrophages and NK cells).[15] All these cells have been implicated in CRC prognosis in different extents.[4-6] Recently, Prizment *et al* suggested that eosinophils may act partially through cytotoxic T-cells in CRC.[9]

Despite the body of evidence placing eosinophils as cells with anti-tumoural activity, their therapeutic potential remains difficult to explore. This is greatly due to the lack of specific activity of eosinophils against tumoural cells.[31] Indeed, attracting eosinophils to the cancer niche and

Main findings
- In univariate analysis, higher stromal EØ scores (*versus* lower stromal EØ scores) associated with better 5-year all-cause and CRC survival (p = 0.0006 and p = 0.001, respectively) - In multivariate analysis, stromal EØ scores (highest *versus* lowest) inversely associated with risk for 5-year all-cause and CRC death (HR 0.61; 95% CI 0.36 - 1.02; p = 0.02 and HR 0.48; 95% CI 0.24 - 0.93; p = 0.01, respectively) as with total follow-up all-cause and CRC-specific death (HR 0.72; 95% CI 0.48 - 1.08; p = 0.04 and HR 0.61; 95% CI 0.34 - 1.12; p = 0.04, respectively) - The association was maintained with statistical significance when stages II and III were combined - In both univariate and multivariate analyses, the highest score of epithelial EØ showed a trend to better outcomes, though without statistical significance
- Both peritumoural and intratumoural EØ associated significantly with progression-free and cancer specific survival (p < 0.001) - Only peritumoural EØ independently associated with progression-free and cancer specific survival (HR 0.75; 95% CI 0.58 - 0.98; p = 0.04 and HR 0.7; 95% CI 0.53 - 0.93; p = 0.01, respectively) - In patients with stage II CRC, the presence of peritumoural EØ (*versus* the absence of peritumoural EØ) independently associated with progression-free and cancer specific survival (HR 0.24; 95% CI 0.07 - 0.87; p = 0.03 and HR 0.25; 95% CI 0.06 - 1.02; p = 0.05, respectively)
- In univariate analysis, EØ count did not associate with cancer-specific survival (HR 1.72; 95% CI 0.89 - 3.35; p = 0.11)
- In univariate analysis, EØ infiltration did not associated with survival ($p \geq 0.3$)
- In univariate analysis, peritumoural EØ directly associated with cumulative survival (p = 0.0008) and inversely associated with local recurrence and distant metastases (p = 0.007 and 0.009, respectively) - In multivariate analysis, peritumoural EØ showed independent prognostic value regarding cumulative survival, additional to TNM staging
- Higher EØ counts independently associated with longer overall survival and relapse-free survival (p = 0.0005 and p = 0.0009, respectively)
- EØ count significantly predicted a good overall survival (p < 0.0001), with a progressive effect of increasing counts on the survival improvement - In univariate analysis, EØ count significantly associated with survival (HR 0.74; 95% CI 0.66 - 0.84; p < 0.0001) - In multivariate analysis, EØ count independently associated with survival (HR 0.81; 95% CI 0.72 - 0.92; p = 0.001) - EØ counts directly associated with survival in Dukes B and C stages (p = 0.03 and 0.002, respectively)
- Overall survival was higher in patients whose tumours had 10 or more EØ - After adjustment for Dukes' stage or treatment, the No. of EØ had no prognostic value
- Survival was significantly greater when more than 30 EØ / mm^2 were counted (p = 0.028)

activating them would most likely lead to detrimental damage of the remaining tissue, in addition to cancer cells.[31] Thus, important lines of research are opening for the future, mainly related to the development of strategies allowing eosinophils to specifically target cancer cells.[31]

Concerning the association between TATE and other prognostic markers of CRC, the results of our review were inconsistent. The limited number of the included studies that associated TATE with overall inflammation or specific cell types and other clinicopathological prognosticators impaired a comprehensive analysis about this topic. The most reliable finding encompassed the association between higher eosinophil counts and lower CRC stage.[9,11,15,20] The association between TATE and such a strong prognostic marker might validate the impact of TATE itself in CRC prognosis. Moreover, this finding suggests that TATE,

Table 4 – Association between TATE and colorectal adenoma-carcinoma progression

First author, year of publication	Results	p value
Kiziltaş, 2008[13] Moezzi, 2000[17]	Hyperplastic polyps < Adenomas	< 0.001
Kiziltaş, 2008[13]	Hyperplastic polyps < Adenocarcinomas	0.022
Cho, 2016[10] Kiziltaş, 2008[13] Moezzi, 2000[17]	Adenomas > Adenocarcinomas	< 0.001
Cho, 2016[10] Kiziltaş, 2008[13]	Low grade dysplasia = High grade dysplasia	> 0.05
	High grade dysplasia > Adenocarcinomas	< 0.001
Moezzi, 2000[17]	Intramucosal carcinoma > Invasive carcinoma	< 0.0001

TATE: tumour associated tissue eosinophilia

namely stromal eosinophils, may prevent CRC progression as stated by Prizment *et al*,[9] which is in line with the decrease of TATE in adenoma-carcinoma progression of colorectal lesions.[10,13,17] In conclusion, these results also point to TATE as a potential immune-evading strategy of CRC.[10] Therefore, in addition to investigating the mechanisms of action of TATE in the context of CRC, it is very important to continue research on the power of TATE in the clinical management of CRC patients. For example, given the decrease of TATE in adenoma-carcinoma progression, TATE has been proposed as a marker of risk for the development of CRC, as well as an indicator for more and tighter surveillance schemes.[13,17] Additionally, TATE may be used as a diagnostic tool to differentiate hyperplastic polyps, adenomatous polyps and serrated adenomas.[13,17] In our review, we only found three articles concerning this topic,[10,13,17] so, clearly, more research is needed in this area.

A major limitation to our review, like in previous ones,[6,28] was the variability between methodologies used in the original research manuscripts which impaired a more meaningful interpretation of the results and the establishment of a threshold above which TATE could predict CRC progression. While the use of immunohistochemistry technique strengthens the identification of tissue eosinophils, an easy, standardized and cost-effective measurement system would be of benefit to enhance future research.[4,9] Furthermore, immunohistochemistry is not free from faults.[29] In fact, TATE was assessed in hematoxylin-eosin stained sections in the majority of the included studies, without compromising the results.[10-13,15-18] Nevertheless, how TATE should be evaluated in terms of location and scoring system, remains unclear. Klintrup *et al* developed an easy and highly reproductive score system – the Klintrup-Mäkinen score – to assess peritumoural overall inflammatory reaction in routinely stained CRC specimens, which proved to be an independent favourable predictor of survival in CRC patients.[44] In that study, higher eosinophilic grade at the invasive margin showed a trend towards a better prognosis.[44] Accordingly, several studies in our series highlighted peritumoural location for TATE evaluation.[11,15,18,20] TATE can be easily evaluated in routinely processed tissues and peritumoural / invasive margins appear to be the most reliable areas for TATE evaluation. Because of all this, TATE seems to meet the standards of a good histopathological prognostic marker as established by Schneider and Langner.[4]

CONCLUSION

According to published studies, we concluded that: 1) current evidence points to TATE as a promising independent prognostic marker in CRC; 2) TATE is inversely associated with the metastatic behaviour of CRC; 3) TATE decreases with adenoma-carcinoma progression of colorectal lesions; and 4) TATE evaluation may have implications on the surveillance schedule and treatments offered to patients diagnosed with CRC. These exciting and promising data call for future research on the role of eosinophils in CRC.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication. Patient consent obtained.

CONFLICTS OF INTEREST

All authors report no conflict of interest.

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